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硕士学位论文

文昌鱼GMNN基因的克隆、序列分析、表征及表达

Cloning, Sequencing, Characterization and Expression of GMNN Gene from Amphioxus

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摘 要

Geminin基因（GMNN）在抑制再复制的进程中是主要的调控者，在细胞周期中的S，G2和早中期时，它负向调节Cdt1p，阻止Mcm2 -7p复合物在复制起点的装配，因此GMNN通过阻止DNA非正常地再复制来维持基因组的忠实性，确保同一个复制起点在一个细胞周期中不会活化两次。在后生动物后期生长中，GMNN在控制细胞分化的同时也控制着细胞的增殖。

GMNN分别抑制青鳉鱼视网膜中Six3和小鼠胚胎中Hox的转录因子，同时在非洲爪蟾中通过抑制Brg-1的活性来维持神经祖细胞处于未分化状态。我们从文昌鱼B. japanicum中克隆得到GMNN的cDNA克隆。运用5’RACE和3’RACE克隆得到GMNN的全长cDNA 为721 bp，经进一步测序验证，然后运用Real time- PCR研究其在胚胎和组织中的表达情况。

文昌鱼GMNN序列编码一个241个氨基酸的蛋白质，它与人GMNN 基因的同源性接近48.02%，与小鼠GMNN基因的同源性达到53.73%，与大鼠GMNN基因的同源性达到62.22%，与原鸡GMNN基因的同源性达到52.4%，与倭黑猩猩GMNN基因的同源性达到62.50 %。通过对文昌鱼的GMNN基因的研究我们发现它是由8个外显子和7个内含子组成，此外，运用实时定量PCR研究GMNN基因的表达情况显示它在卵巢中高表达，而在精巢没有得到高表达。文昌鱼GMNN基因的克隆和表达结果显示文昌鱼该基因是一个编码相对短的蛋白的小基因，而与其他脊椎动物不同的是该基因并不在增生组织表达。

关键词：文昌鱼；GMNN基因；Cdt1p；Mcm2 -7p复合物

Abstract

Geminin gene (GMNN) is a central regulator of the process that inhibits re-replication. During S, G2, and early M phases of the cell cycle, it negatively regulates the Cdt1p and prevents them from loading the Mcm2–7p complex to the origins of replication. GMNN thus maintains the integrity of the genome by preventing abnormal re-replication of DNA. It ensures that, the same origin of replication is not activated twice within the same cell cycle. GMNN gene controls differentiation as well as cell proliferation during late metazoan development.

It inhibits both Six3 and Hox transcription factors in Medaka retina and the mouse embryo, respectively, and maintains an undifferentiated state in neural progenitor cells of Xenopus laevis by antagonizing Brg-1 activity.

We have cloned a cDNA clone encoding GMNN from the amphioxus, B. japanicum. The721 bp full-length GMNN gene cDNA was cloned using 5'RACE and 3' RACE and further confirmed by nucleotide sequencing and then expressed using Real time RT-PCR.

The amphioxus GMNN gene sequence encodes a protein of 241 amino acids which displays approximately 48.02% homology with human GMNN gene, 53.73% with the mouse GMNN gene, 62.22% with Rattus norvegicus GMNNgene, 52.4% with the Gallus gallus GMNN gene and 62.50 % with Pan troglodytes.

By studying GMNN gene sequence organization we found that it is made of 8 exons and 7 introns. Furthermore, the expression pattern of GMNN gene with Real Time RT-PCR reveled that GMNN gene is highly expressed in the ovary but not in the testis. Our results on cloning and expression the GMNN gene in amphioxus revealed that the amphioxus GMNN gene is a short gene encoding a relatively short protein but unlike in other vertebrates, is not expressed in proliferative tissues.

Keywords: Amphioxus; GMNN gene; Cdt1p; Mcm2–7p complex

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